SUPPORTING ONLINE MATERIAL

Materials and methods

Protein Purification

Cytoplasmic dynein-dynactin from brains of transgenic mice expressing green fluorescent protein (GFP)-labeled dynamitin was purified by microtubule-affinity ATP extraction and sucrose density gradient fractionation as previously described (*1*). Fraction 6 containing dynein-dynactin-GFP (Fig. S1C) was supplemented with 10 µM ATP, 1 mM dithiothreitol (DTT), and 25% sucrose and stored in liquid nitrogen. The integrity of the complex was assessed by SDS-PAGE and western blot probing for dynein heavy chain (Polyclonal, Santa Cruz Biotechnology Inc., Santa Cruz, CA), dynein intermediate chain (MAB 1618, Chemicon, Temecula, CA), $p150^{Glued}$ (Monoclonal, BD transduction laboratories, San Jose, CA), dynamitin ((Monoclonal, BD transduction laboratories, San Jose, CA), and kinesin heavy chain (MAB 1614, Chemicon). GFP-labeled recombinant kinesin (K560-GFP; the expression plasmid was a gift from R. Vale, University of California, San Francisco, CA) was purified from bacteria using polyhistidine-tag based affinity purification and stored at – 80 °C. Recombinantly expressed tau isoforms and truncated versions (expression plasmids were a gift from E. Mandelkow and J. Biernat, Max Planck Unit for Structural Molecular Biology, Hamburg, Germany) were purified from bacteria according to Barghorn et al. (*2*) and stored at – 80 °C in motility assay buffer (10 mM sodium-PIPES, 50 mM potassium acetate, 4 mM $MgSO_4$, 1 mM EGTA, pH = 7). Tau protein concentration was determined by densitometry on Immobilon (Millipore, Bedford, MA) blots using commercially available tau40 and tau23 (Sigma, St.

Louis, MO) as standards. Purified tau proteins were fluorescently labeled at cysteine residues using Alexa Fluor 546 C_5-maleimide (Invitrogen, Eugene, OR) in motility assay buffer. The degree of labeling was estimated to be about 60% by spectrophotometric analysis. Microtubule binding assays were conducted by co-incubating 50 nM taxol-stabilized microtubules with various concentrations of either unlabeled tau or Alexa-labeled tau in motility assay buffer at 25 °C for 20 min. Samples were then centrifuged at 35,000 g for 20 min and the supernatant and pellet fractions analyzed by western blotting using polyclonal anti-tau antibody (Abcam, Cambridge, MA).

Single molecule motility assays

In vitro motility assays were conducted in flow chambers assembled from a glass slide and silanized cover slip attached using double-sided adhesive tape (chamber volume ~ 15 µl). 50 nM microtubules were incubated with varying concentrations of tau for 20 minutes at 25 °C and then introduced in a flow chamber coated with 0.08 % monoclonal anti-β tubulin antibody (Tub 2.1 clone, Sigma, St. Louis, MO) and blocked with 5% Pluronic F-127 (Sigma). Motor protein diluted in motility assay buffer supplemented with 1 mM ATP, 1 mg/ml bovine serum albumin, an oxygen scavenging system containing glucose oxidase, catalase and glucose (*3*), 10 mM DTT and the appropriate concentration of tau was subsequently flowed into the chamber. The addition of tau in the motility mix is necessary to maintain constant tau concentration and to avoid tau dissociation during the 10-15 min observation period. Omission of this step caused progressive dissociation of tau from the microtubule surface and consequently no apparent effect of tau on run lengths as reported by Seitz et al. (*4*). Single dynein-dynactin-GFP or kinesin-GFP

molecules were visualized at 25 °C using total internal reflection fluorescence (TIRF) microscopy outfitted on an inverted microscope (Nikon TE-2000U). TIRF excitation was achieved using a 488-nm Argon-ion laser to visualize GFP and a 532-nm diode-pumped solid-state laser (CrystaLaser, Reno, NV) to visualize rhodamine and Alexa546. Images were captured with a back-illuminated electron multiplier-CCD camera (Cascade-512B, Photometrics, Tucson, AZ) using burst mode at 4 frames per second. Control (0 nM tau) experiments were always performed in parallel on the same day using the same motor preparation.

Data Analysis

The motions of single dynein-dynactin-GFP or kinesin-GFP motors were analyzed using kymographs generated with the "Multiple Kymograph" plug-in for ImageJ submitted by J. Rietdorf and A. Seitz (European Molecular Biology Laboratory, Heidelberg, Germany). A processive run is defined here as unidirectional motion over at least 250 nm. For the experiments using Alexa-tagged tau, pauses were defined as events in which the motor was stationary for at least 10 frames (~ 3 sec) and a reversal was defined as an event in which a processive run in one direction was followed by a processive run in the opposite direction. To compare the movement of motors within tau patches to movement outside tau patches, the boundaries of a fluorescent tau patch were assigned as half-maximal fluorescence at the edge. Since dynein moves bidirectionally, its run length was measured as the sum of the processive run lengths in both directions. Fitting of distributions of single molecule events was conducted using Kaleidagraph (Synergy

Software, Reading, PA) and statistical significance was determined using the Student's t-test.

Supplementary figure legends

Fig. S1. Purification of motor proteins and tau isoforms.

(A) The upper panel is a coomassie blue-stained blot of Alexa-tagged tau40, tau23, K35 and K33 versions of tau protein. As previously reported, tau isoforms and truncations migrate anomalously (*2*). The lower panel is a fluorescence image of the same blot showing Alexa546 labeling of the proteins. Molecular weight markers are indicated to the left in kDa. (B) Coomassie blue-stained blot of purified recombinant kinesin (K-GFP). (C) Western blot analysis of sucrose density gradient fractions of mouse brain lysate. The position of dynein heavy chain (DHC), p150^{Glued} (p150), kinesin heavy chain (KHC), dynein intermediate chain (DIC), and dynamitin (p50) are indicated by arrows. Fraction 6 was used in our experiments because it is enriched for dynein-dynactin and lacking kinesin.

Fig. S2. Microtubule binding of unlabeled and labeled tau.

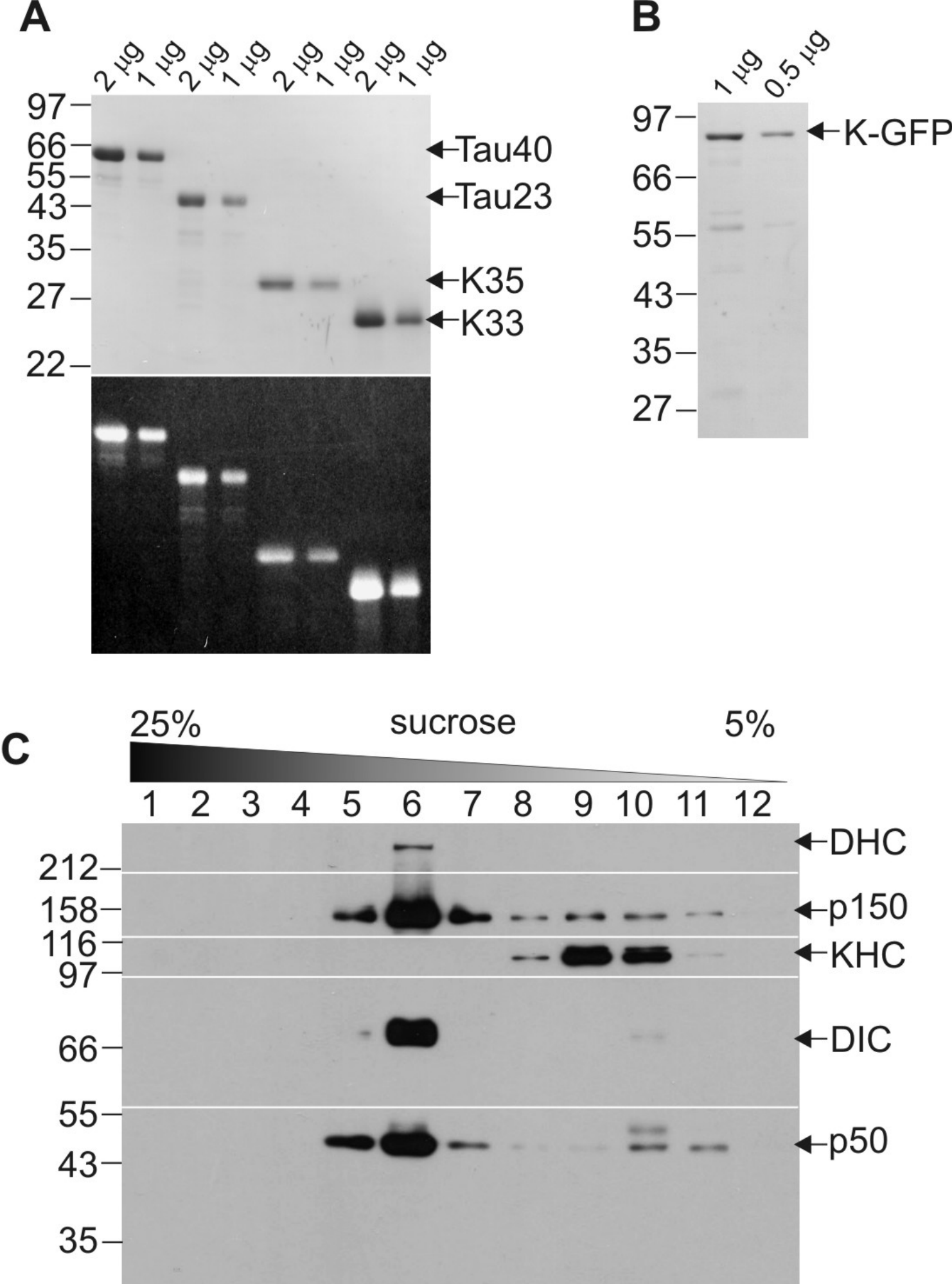
The graphs show the percent microtubule bound tau at various concentrations of either unlabeled tau (white bars) or Alexa-labeled tau (red bars). The results are the mean ± SEM of three independent experiments.

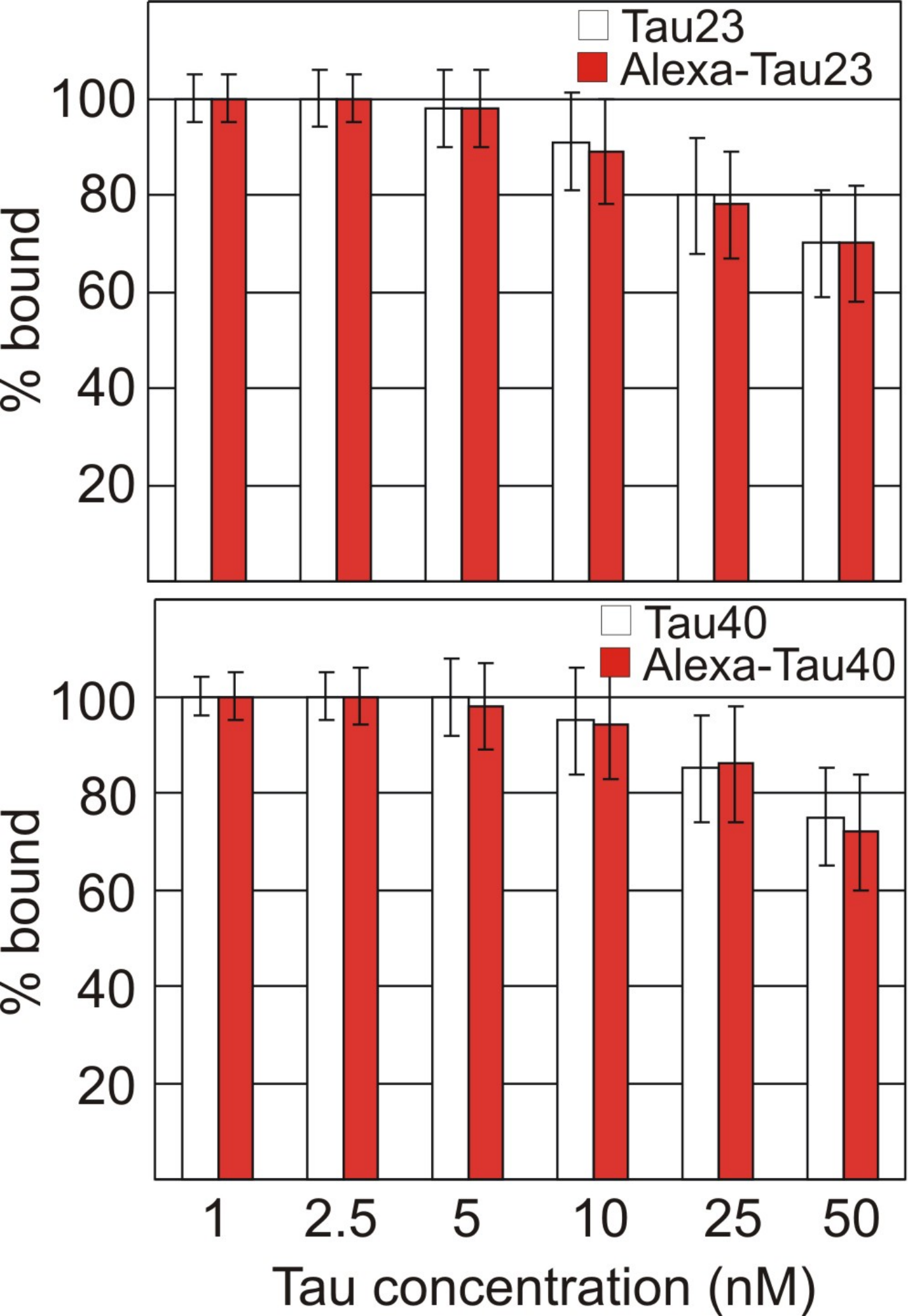
Fig. S3. Relationship between local tau concentration and kinesin-GFP or dynein-dynactin-GFP binding.

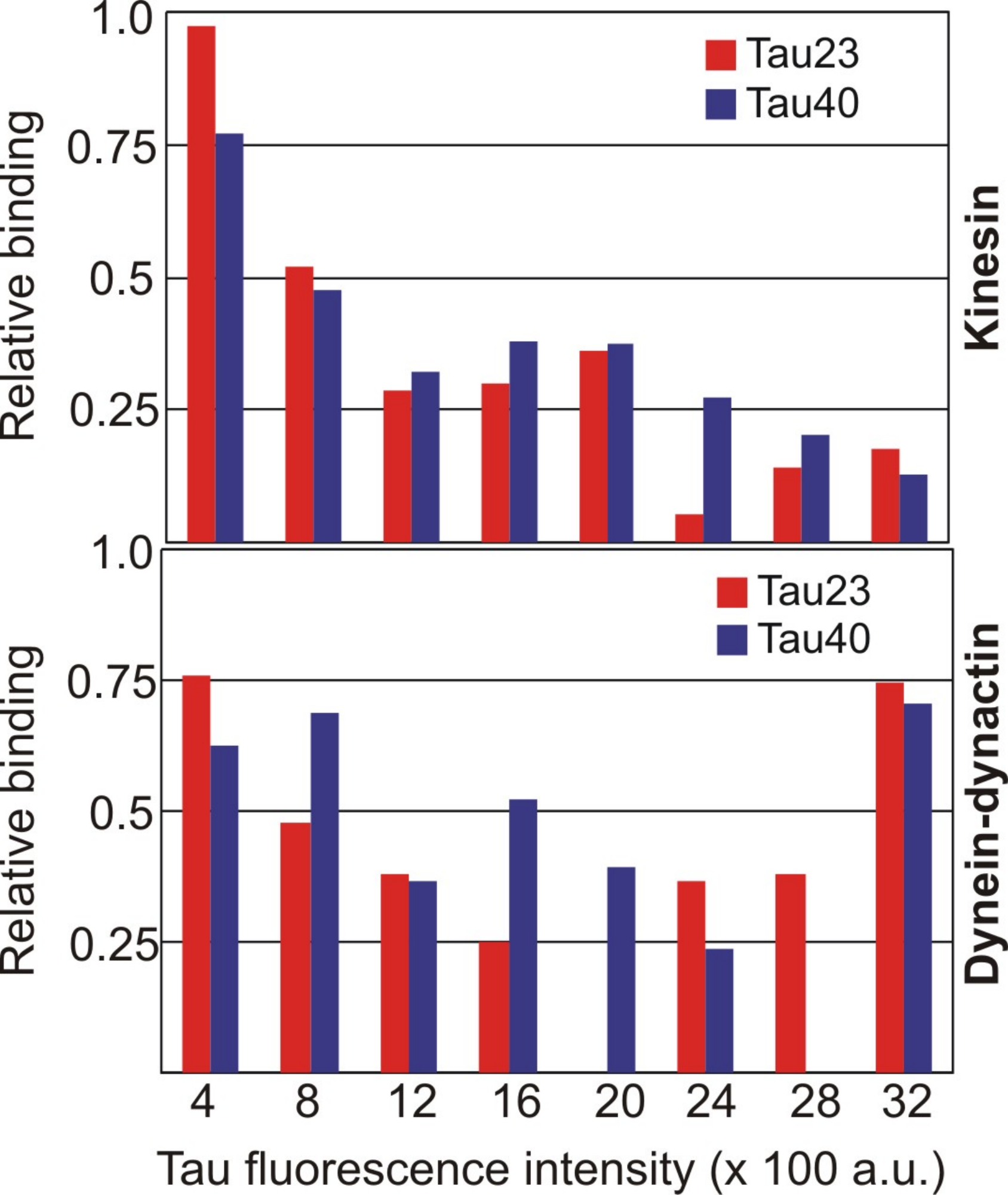
The graphs show the distribution of the relative number of binding events of either kinesin or dynein-dynactin ($n \geq 100$ for each case) as a function of the Alexa-tau23 (red

bars) or Alexa-tau40 (blue bars) fluorescence intensity at the binding site. The data are normalized for the distribution of tau fluorescence intensity on the microtubule surface.

Fig. S4. Microtubule decoration pattern of Alexa-labeled tau proteins. Representative images of microtubules decorated with Alexa-tagged tau40, tau23, K35, and K33. Note the patchy pattern of microtubule binding of tau. The power density spectra of the fluorescence intensity profiles of Alexa-tau along microtubules show a low spatial frequency component at 0.1-0.6 μm^{-1}, consistent with clusters of tau on the microtubule surface. In contrast, the power density spectra of fluorescence along microtubules randomly labeled by copolymerizing low levels of rhodamine-tubulin (Rhod) are nearly flat below spatial frequency 1.2 μm^{-1}.


A
2 µg
1 µg
2 µg
1 µg
2 µg
1 µg
2 µg
1 µg
97
66
55
43
35
27
22
Tau40
Tau23
K35
K33
B
1 µg
0.5 µg
97
66
55
43
35
27
K-GFP
C
25%
sucrose
5%
1 2 3 4 5 6 7 8 9 10 11 12
212
158
116
97
66
55
43
35
DHC
p150
KHC
DIC
p50


Tau23
Alexa-Tau23
100
80
60
40
20
% bound
Tau40
Alexa-Tau40
100
80
60
40
20
% bound
1
2.5
5
10
25
50
Tau concentration (nM)


Relative binding
1.0
0.75
0.5
0.25
Tau23
Tau40
Kinesin
Relative binding
1.0
0.75
0.5
0.25
Tau23
Tau40
Dynein-dynactin
4
8
12
16
20
24
28
32
Tau fluorescence intensity (x 100 a.u.)

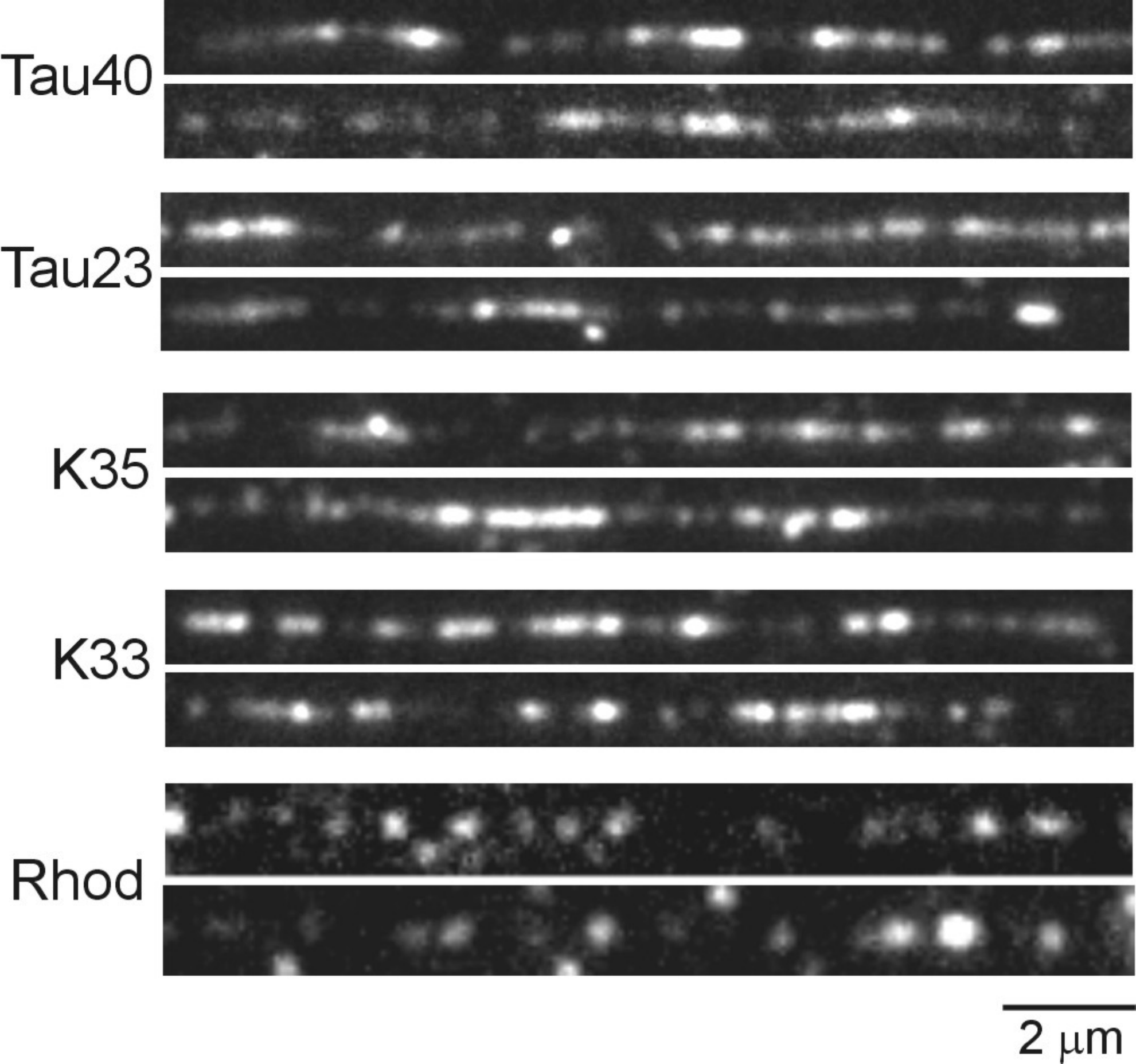

Tau40
Tau23
K35
K33
Rhod
2 μm

Table S1. Velocity of kinesin and dynein at various tau concentrations

	Kinesin	**Dynein**
	Velocity (nm/s)	Velocity (nm/s)
0 nM tau23	444 ± 18	575 ± 18
1 nM tau23	420 ± 75	595 ± 31
10 nM tau23	442 ± 23	597 ± 55
100 nM tau23	N/A	557 ± 49
0 nM tau40	421 ± 43	599 ± 47
1 nM tau40	453 ± 36	611 ± 38
10 nM tau40	438 ± 82	604 ± 62
100 nM tau40	458 ± 60	582 ± 53

The data show the mean velocities ± SEM of ≥ 100 molecules for 0-10 nM tau and ~ 30 molecules for 100 nM tau at 1mM Mg-ATP.

Supplementary References

1. J. L. Ross, K. Wallace, H. Shuman, Y. E. Goldman, E. L. F. Holzbaur, *Nat. Cell Biol.* **8**, 562 (2006).

2. S. Barghorn, J. Biernat, E. Mandelkow, *Methods Mol. Biol.* **299**, 35 (2005).

3. Y. Harada, K. Sakurada, T. Aoki, D. D. Thomas, T. Yanagida, *J. Mol. Biol.* **216**, 49 (1990)

4. A. Seitz *et al.*, *EMBO J.* **21**, 4896 (2002).

Movie legends

Movie S1. Response of kinesin to encounters with Alexa-tau23.

Pseudo-colored movie showing the Alexa-tau23 patches along a microtubule in red, and kinesin-GFP molecules in green. The closed white arrowheads mark kinesin molecules that detach upon encountering tau. The open white arrowhead marks a kinesin molecule that binds within a tau patch and the white arrow marks a kinesin molecule that pauses at a tau patch (indicated by the arrow turning yellow). Scale bar = 2 μm.

Movie S2. Response of kinesin to encounters with Alexa-tau40.

Pseudo-colored movie showing the Alexa-tau40 patches along a microtubule in red, and kinesin-GFP molecules in green. The white arrowheads mark kinesin molecules that detach upon encountering tau. The estimated number of tau molecules at the peak fluorescence intensity of the tau patch is five. Scale bar = 2 μm.

Movie S3. Response of dynein-dynactin to encounters with Alexa-tau23.

Pseudo-colored movie showing the Alexa-tau23 patches along a microtubule in red, and dynein-dynactin-GFP molecules in green. The white arrowheads mark dynein-dynactin molecules that reverse direction upon encountering tau. Scale bar = 2 μm.

Movie S4. Response of dynein-dynactin to encounters with Alexa-tau40.

Pseudo-colored movie showing the Alexa-tau40 patches along a microtubule in red, and dynein-dynactin-GFP molecules in green. Dynein-dynactin motility is relatively insensitive to the presence of tau40 on the microtubule surface. Scale bar = 2 μm.

Movie S5. Kinesin motility on bare microtubules.

Movement of single kinesin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the absence of tau23. Kinesin travels unidirectionally, as seen for a molecule marked by the yellow arrowhead. Scale bar = 3 μm.

Movie S6. Effect of 10 nM tau23 on kinesin motility.

Movement of single kinesin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the presence of 10 nM tau23. Kinesin run length is substantially decreased by 10 nM tau23, as seen for a molecule marked by the yellow arrowhead. Scale bar = 3 μm.

Movie S7. Effect of 100 nM tau23 on kinesin motility.

Movement of single kinesin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the presence of 100 nM tau23. Processive motility of kinesin is completely inhibited by 100 nM tau23. Scale bar = 3 μm.

Movie S8. Dynein-dynactin motility on bare microtubules.

Movement of single dynein-dynactin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the absence of tau23. Note the robust bidirectional motility of dynein-dynactin, as seen for a molecule marked by the yellow arrowhead. Scale bar = 3 μm.

Movie S9. Effect of 10 nM tau23 on dynein-dynactin motility.

Movement of single dynein-dynactin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the presence of 10 nM tau23. Dynein motility is marginally affected by 10 nM tau23, as seen for a molecule marked by the yellow arrowhead. Scale bar = 3 μm.

Movie S10. Effect of 100 nM tau23 on dynein-dynactin motility.

Movement of single dynein-dynactin-GFP molecules along rhodamine-labeled microtubules (shown in the first frame) in the presence of 100 nM tau23. Dynein is capable of processive motility in the presence of 100 nM tau23, as seen for a molecule marked by the yellow arrowhead. Scale bar = 3 μm.